

SENSOR ARRAY INTEGRATED ELECTROCHEMICAL CHIP, METHOD OF  
FORMING SAME, AND ELECTRODE COATING

**[0001]** The present invention relates to sensor array integrated electrochemical chips, methods of forming same, and electrode coatings.

**[0002]** Electrochemical sensors are useful for detecting the presence of, or measuring the concentration of, a target chemical or biochemical substance in a fluid.

**[0003]** A typical electrochemical sensor has a sensing electrode (also known as working electrode or measuring electrode), and one or both of a counter electrode (also known as auxiliary electrode) and a reference electrode. The electrodes are immersed in the fluid containing the target substance during operation. A key process in an electrochemical reaction is the transfer of electrons between the working electrode surface and molecules in the interfacial region (either in the fluid or immobilized at the electrode surface). An electrical signal can be detected if the working electrode is exposed to the target substance. The signal arises due to either a change in potential at the electrode, or a flow of electrons (current) through the electrodes which is generated in response to an imposed voltage signal on the electrodes, as a result of the reduction-oxidation reactions, known as redox reactions, occurring at the electrode surfaces.

**[0004]** An electrode of a sensor may be covered by a coating to control the properties, selectivity and sensitivity of the sensor. For example, it is sometimes desirable to control the electrical resistance at the electrode-fluid interface. The resistance at the interface affects the current response of the electrode, because it affects the permeability of electrolytes that reach the electrode, and consequently the signal/noise ratio. In this regard, metal supported bilayer lipid membranes (s-BLM) have been used as a coating on electrodes. See e.g., Tien *et al.*, "Supported Bilayer Lipid Membranes as Ion and Molecular Probes", *Analytical Sciences*, (1998), vol. 14, p. 3. However, known s-BLM coatings only provide a limited increase in electrical resistance. Further, with known s-BLM coatings, it is difficult to obtain a stable specific resistance, as the resistance of the coating material is not easily controllable and the formed coating can be damaged due to rugged laboratory handling.

**[0005]** Sensor array integrated devices are useful as they are compact and can be used to simultaneously analyze the same component at different measuring points or different components of a sample. A number of techniques have been used to form

electrochemical sensor array integrated devices. For example, U.S. patent 6,315,940 to Nisch *et al.* discloses a microelement device having a base plate and a cover plate, wherein the cover plate has multiple microcuvettes, each of which encloses a sensing electrode formed inside a microcuvette of the cover plate, or on top of the base plate, or in a third plate sandwiched between the cover plate and the base plate. These existing techniques, however, have drawbacks, such as a relatively complicated process for the fabrication of the integrated devices.

**[0006]** Therefore, there remains a need for an improved electrode coating, and improved approaches to forming electrodes and sensor array integrated electrochemical chips.

## SUMMARY OF THE INVENTION

**[0007]** A sensor array integrated electrochemical chip is provided wherein the chip has an array of electrodes. At least one of the electrodes may be covered by a coating doped with a ferrocene compound. The array may be formed on a base plate bonded to a cover plate having an opening such that the array is within a cavity defined by the base plate and the cover plate. Conducting lines for connecting the electrodes to electrochemical instruments may be formed on the same surface of the base plate on which the electrodes are formed.

**[0008]** In accordance with one aspect of the invention, there is provided a sensor array integrated electrochemical chip comprising an array of electrodes, at least one electrode of the array of electrodes being covered by a coating doped with a ferrocene compound.

**[0009]** In accordance with another aspect of the invention, there is provided a method of forming an electrochemical chip, comprising forming a first plate by depositing a conducting layer on a first support and etching the conducting layer to form an electrode array; forming a second plate by etching an opening in a second support; and bonding the second plate to the first plate such that the first plate and the second plate define a cavity, with the electrode array being within the cavity. The opening may be a window or a depression. The method may further comprising covering at least one electrode of the electrode array with a coating doped with a ferrocene compound. The method may further comprising oxidizing the ferrocene compound.

**[0010]** In accordance with yet another aspect of the invention there is provided a method of forming an electrochemical chip comprising forming a metal array; and covering at least some elements of the array with a supported bilayer lipid membrane doped with a ferrocene compound.

**[0011]** In accordance with still another aspect of the invention there is provided a method of using a ferrocene compound as a dopant in an electrode coating.

**[0012]** In accordance with yet another aspect of the invention there is provided a sensor array integrated electrochemical chip, comprising a first plate having an array of electrodes thereon and a second plate having an opening, the second plate bonded to the first plate so that the first plate and the second plate define a cavity, with the array of electrodes being within the cavity. The opening may be a window or a depression. The first plate may have a plurality of conducting lines formed on a same surface of the first plate on which the array of electrodes is formed, each of the conducting lines extending from one of the electrodes outwardly beyond a periphery of the array.

**[0013]** Other aspects, features and advantages of the invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0014]** In the figures, which illustrate exemplary embodiments of the invention,

**[0015]** **FIG. 1** is a schematic perspective view of a sensor array integrated electrochemical chip;

**[0016]** **FIG. 2** is a schematic partial cross-sectional view of the electrochemical chip of **Fig. 1**;

**[0017]** **FIG. 3A-3C** schematically illustrate an approach to forming the base plate of **FIG. 1**;

**[0018]** **FIG. 4A-4D** schematically illustrate an approach to forming the cover plate of **FIG. 1**;

**[0019]** **FIG. 5** is a schematic partial cross-sectional view of another sensor array

integrated electrochemical chip;

[0020] FIG. 6 is a schematic partial cross-sectional view of a cover plate; and

[0021] FIG. 7 is a partial plan view of an electrode array on a base plate.

#### DETAILED DESCRIPTION

[0022] When used herein:

- “array of electrodes” means at least two electrodes formed in any pattern. The electrodes can be either interconnected or independently wired.
- “sensor array” means an array of sensors which can consist of identical or different sensors.
- “bonded” means held together, either chemically or mechanically, or otherwise.
- “ferrocene compound” means a chemical compound containing a ferrocene group. A ferrocene group has the chemical formula  $C_5H_5FeC_5H_4-$ . Examples of ferrocene compounds include ferrocene ( $C_5H_5FeC_5H_5$ ) and benzoylferrocene ( $C_5H_5FeC_5H_4COC_6H_5$ ).

[0023] In overview, a sensor array integrated electrochemical chip may have an array of electrodes. At least one of the electrodes may be covered by a coating doped with a ferrocene compound, such as a supported bilayer lipid membrane (s-BLM) doped with benzoylferrocene. The electrodes may be formed on a base plate, which is bonded to a cover plate having an opening such that the array is within a cavity defined by the base plate and the cover plate. The opening of the cover plate may be a window or a depression. The electrodes may be interconnected or independently wired. The electrodes and the connecting lines may be formed on the same surface of the base plate.

[0024] The ferrocene doped coating has high electrical resistance. Thus, the resistance at the coated electrodes is higher compared to un-coated electrodes or electrodes coated with a conventional undoped s-BLM. The increase in electrical resistance of the coating covering the electrodes can be controlled by both the doping concentration and the degree of oxidization of the ferrocene compound. In this regard, it

has been discovered that oxidizing the doped ferrocene compound increases resistance. With an appropriate resistance, the signal-to-noise ratio of the sensor can be increased.

**[0025]** Figs. 1 and 2 illustrate schematically an electrochemical chip **10** having a base plate **12** and a cover plate **14**. An array of electrodes **16** is formed on base plate **12**. Cover plate **14** has an opening, window **17**. Base plate **12** and cover plate **14** together define a cavity, reaction chamber **18**, such that the array of electrodes **16** is within reaction chamber **18**.

**[0026]** Optionally, one or more cantilevers **20** can be provided on cover plate **14** as support for external electrodes (not shown), such as an external counter or reference electrode, or both. Each external electrode may be inserted into an opening **22** in a cantilever **20** and extended into reaction chamber **18**.

**[0027]** Electrodes **16** may all be working electrodes, or may include one or more counter electrodes and/or reference electrodes. Each electrode **16** may be individually electrically controlled through contact holes **24** on printed circuit board (PCB) **26**. PCB **26** provides electrical input/output connections to external electrical and/or electronic instruments. As will be understood by a person skilled in the art, bond pads and conducting lines (also known as “runners”) are typically used for connecting the electrodes to contact holes **24**. External instruments may be connected to the electrodes via contact holes **24**. Alternatively, the bond pads and conducting lines could directly connect the electrodes to external instruments. The bond pads and the conducting lines can also be formed on the base plate **12**. However, for clarity, the bond pads and conducting lines connecting electrodes **16** to contact holes **24** are not shown in Fig. 1 (but shown in Fig. 7).

**[0028]** Electrochemical chip **10** may have various sizes and shapes suitable for particular applications. For example, electrochemical chip **10** may have a chip size varying from 1 x 1 cm to 2 x 2.25 cm and a chamber area varying from 6 x 6 mm to 2 x 44 mm.

**[0029]** While an array of 5 x 5 electrodes is shown in FIG. 1, the array of electrodes **16** may have any suitable pattern or number of electrodes depending on the application. Electrodes **16** may have various shapes, such as square, rectangular, circular, ovoid, and the like. Electrodes **16** may also have various sizes and can be made very small,

such as less than 90  $\mu\text{m}$  in length and width. Diameters from 10  $\mu\text{m}$  to 90  $\mu\text{m}$  have been tested and were found to be suitable. Typically, each working electrode may have a surface area in the range of  $1 \times 10^{-7}$  to  $1 \times 10^{-4} \text{ cm}^2$  or less. Smaller sizes may be preferable in many biochemical applications. Electrodes **16** may be evenly or unevenly spaced with various inter-electrode distances depending on the application. For example, inter-electrode distances in the range of 10 to 100  $\mu\text{m}$  were found suitable in an exemplary electrochemical chip. The pixel sizes of chip **10** may also vary. Pixels with a length of 0.25 mm were found suitable in an exemplary electrochemical chip.

**[0030]** As illustrated in **Fig. 2**, base plate **12** may be bonded to cover plate **14** by a bonding material **38**.

**[0031]** Base plate **12** includes base wafer **30**, first insulation layer **32**, conducting layer **34**, and second insulation layer **36**. Wafer **30** may be made of silicon, or other suitable material such as glass, plastic, polymer sheet, ceramic and semiconductor materials. Insulation layers **32** and **36** may be made of the same or different materials. Suitable material for insulation layers **32** and **36** include silicon dioxide, silicon nitride, and other suitable organic and inorganic materials. For use in biochemical applications, the exposed materials (uncovered portions of wafer **44** and layers **32** and **36**) should be compatible with the intended electrolytes and biological or biochemical testing solutions. Insulation layers **32** and **36** should be thick enough to provide sufficient insulation. Each of insulation layers **32** and **36** may have a thickness in the range of 0.1 to 5  $\mu\text{m}$ . Conducting layer **34** may comprise suitable conducting material, such as Cr, Au and Ti, and may itself be layered. For example, conducting layer **34** may be formed by a layer of Au on top of a layer of Cr or Ti. Electrode array **16** is formed from conducting layer **34**. The aforesaid conducting lines for connecting the electrodes to external instruments may also be formed from conducting layer **34**. At least one electrode **16** may be covered by a coating **28** doped with a ferrocene compound, such as a benzoylferrocene doped s-BLM. If desired, some or all of electrodes **16** may be covered by a ferrocene doped coating.

**[0032]** Cover plate **14** includes wafer **44** and mask layers **40**, **42**, **46**, and **48**. Like wafer **30**, wafer **44** may be made of silicon or other suitable material. Mask layers **40**, **42**, **46**, and **48** are deposited on wafer **44** to mask wafer **44** during etching. If wafer **44** is to be etched with a wet chemical method, two mask layers may be deposited on each side of wafer **44**, as shown in **FIG. 2**. The inner mask layers **42** and **46** may be thermal

oxide layers and the outer mask layers **40** and **48** may be silicon nitride layers deposited by low pressure chemical vapour deposition. Layers **42** and **46** may be 0.03 to 1  $\mu\text{m}$  thick. Layers **40** and **48** may be 0.1 to 2  $\mu\text{m}$  thick. If wafer **44** is to be etched with a dry etching method, a single mask layer on each side may be sufficient. The single mask layer may be formed with materials such as photoresist, silicon oxide, silicon nitride, and any other materials suitable for dry etching. One or more of mask layers **40**, **42**, **46**, and **48** may be removed after etching. However, to simplify the fabrication process, the mask layers may be kept intact.

**[0033]** Example approaches to forming base plate **12** and cover plate **14** are illustrated in **Fig. 3A to 3C** and **4A to 4D**.

**[0034]** Referring to **Fig. 3A**, insulation layer **32** is deposited onto silicon wafer **30** to form a support. Conducting layer **34** is next deposited onto insulation layer **32**. Any suitable deposition technique can be used.

**[0035]** Referring to **Fig. 3B**, conducting layer **34** is then patterned and etched to form electrode array **16**, bond pads, and circuit lines connecting each electrode to a bond pad. Standard semiconductor microfabrication techniques such as lithograph techniques may be used.

**[0036]** Referring to **Fig. 3C**, insulation layer **36** is next deposited onto conducting layer **34**. Layer **36** is then etched to expose electrode array **16** and bond pads. Layer **36** can be etched with any suitable etching technique such as plasma dry etching or wet chemical etching. Layer **36** is not etched away in the area around a periphery of the electrode array **16** where the two bonded plates will be in contact. As illustrated in **Fig. 2**, cover plate **14** will be bonded to base plate **12** at the remaining insulation layer **36** which abuts cover plate **14**.

**[0037]** Referring to **Fig 4A**, a support for forming cover plate **14** is formed by depositing, sequentially, mask layers **42** and **46** and then mask layers **40** and **48** on both sides of wafer **44**. Wafer **44** may be made of silicon. The insulation layers may be formed using a low pressure chemical vapor deposition technique.

**[0038]** Referring to **Fig. 4B**, the window area for reaction chamber **18**, the areas for bond pads, and areas for cantilevers **20** are etched out from layers **40**, **42**, **46** and **48**, using standard semiconductor microfabrication techniques such as lithograph

techniques, to expose wafer **44** in these areas.

**[0039]** Referring to **Fig. 4C**, wafer **44** is etched from the front side (top side in the figures), which will face away from base plate **12** when cover plate **14** is bonded to base plate **12**. More than 10  $\mu\text{m}$  thick of wafer **44** may be etched away within the open areas. Referring to **Fig. 4D**, wafer **44** is then etched through from the backside (bottom side in the figures), which will face base plate **12** when cover plate **14** is bonded to base plate **12**. The side exterior of wafer **44** may also be etched to expose the bond pads. To etch wafer **44**, techniques such as KOH wet chemical etching or silicon dry etching may be used.

**[0040]** As noted above, one or more of mask layers **40**, **42**, **46**, and **48** may be removed after etching.

**[0041]** Returning to **Fig. 2**, base plate **12** and cover plate **14** can then be bonded together, with window **17** of cover plate **14** over electrode array **16**, using techniques such as taught by U.S. patent 6,503, 847 to Chen *et al.* ("Chen") issued Jan. 7, 2003, the contents of which are incorporated herein by reference. In particular, diluted polydimethylsiloxane (PDMS) solutions may be prepared as taught in Chen. The surface of layer **40** (or the backside of wafer **44** if mask layers **40** and **42** have been removed) is then spin coated with PDMS. The PDMS coating may have a thickness in the range of 1 to 500  $\mu\text{m}$ . During the coating process, the front side of wafer **44** may be laminated with polymer film to prevent them from being coated by PDMS. The PDMS may be pre-cured to half curing before cover plate **14** is aligned to base plate **12** and the two plates are pressed toward each other until the PDMS is fully cured. As can be understood, although not shown in **Fig. 2** (for clarity), the backside of cover plate **14** and the inner surfaces of window **17** are covered with a layer of PDMS after bonding. Conveniently, the PDMS layer is a biocompatible material so that even if wafer **30** and the mask layers are not biocompatible, chip **10** may still be biocompatible.

**[0042]** As can be appreciated, more than one pair of base and cover plates can be formed simultaneously from two sheets of wafer. If more than one pair is formed, individual pairs can be cut out after the PDMS is fully cured, such as by dicing. Each pair may then be wire bonded to a PCB.

**[0043]** As mentioned, one or more electrodes **16** are covered by a ferrocene compound doped coating, such as a benzoylferrocene doped s-BLM. The coating may



be deposited on an electrode **16** in various ways. An exemplary procedure for covering an electrode **16** with a benzoylferrocene doped s-BLM is as follows:

- Dissolve benzoylferrocene and dimyristoyl L- $\alpha$ -phosphatidylcholine (DMPC) in analytical grade chloroform to prepare a lipid solution wherein the concentrations of benzoylferrocene and DMPC are in the range of 0.1 to 10mg/ml. For example, they may respectively be 1 mg/ml and 2 mg/ml.
- Clean electrochemical chip **10**. For example, sonicate chip **10** successively in alcohol and de-ionized water for 5 minutes each and then dry chip **10** in air;
- Apply the lipid solution onto the surface of the electrode in small quantities, such as drop 20  $\mu$ l aliquots of the lipid solution with a microsyringe;
- Evaporate chloroform on the electrode gradually in air at room temperature;
- Transfer 1 ml of phosphate buffer solution (PBS) onto the lipid-coated electrode **16**, wherein the buffer solution may contain 8 g/l NaCl, 0.2 g/l KCl, 1.44 g/l  $\text{Na}_2\text{HPO}_4$ , 0.24 g/l  $\text{KH}_2\text{PO}_4$ , with a pH value of 7.4. The buffer solution can also be any suitable neutral aqueous solution in which the lipid bilayer membrane is stable.

**[0044]** Other suitable doping procedures, such as absorption and diffusion doping methods may also be employed. It will be appreciated, however, that the doping process should not negatively affect the s-BLM properties.

**[0045]** As can be appreciated, a benzoylferrocene doped s-BLM will form spontaneously (by molecular self-assembling) in the PBS solution on the electrode.

**[0046]** Test results have shown that the electrical resistance of an s-BLM coating is higher when doped with benzoylferrocene than without doping. Test results also show that electrical resistance can be further increased if the doped benzoylferrocene is oxidized.

**[0047]** Benzoylferrocene in the coated s-BLM can be oxidized by subjecting the electrodes **16** to cyclic potential changes for example from  $-0.3$  to  $+0.8$  V when they are immersed in an electrolytic solution such as a PBS containing potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), whereby redox reactions occur on the coating.

**[0048]** Whether or not benzoylferrocene in the coating has been oxidized can be readily tested by examining the electrode's current response to redox reactions. When the current response is small, it has been oxidized. If the current response is large, then it has not been fully oxidized.

**[0049]** Conveniently, the electrical resistance at electrodes **16** can be controlled by controlling the degree of oxidization of benzoylferrocene in the s-BLM coating. Furthermore, test results show that the oxidization of benzoylferrocene is irreversible. That is, once oxidized, the benzoylferrocene will remain oxidized, thus providing a stable electrical resistance at the electrode interfaces. (However, if the benzoylferrocene is not fully oxidized, it may become further oxidized if the potential applied to the electrodes is higher than the oxidation potential.)

**[0050]** The resistance of the coating can also be controlled by adjusting the dopant concentration.

**[0051]** Other ferrocene compounds may also be used to dope the s-BLM coating. For example, ferrocene ( $C_5H_5FeC_5H_5$ ) or 1,1'[(4,4'-Bipiperidine)-1,1'-diylidicarbonyl]-bis[1'-(methoxycarbonyl)ferrocene] may be used.

**[0052]** Further, s-BLM may be replaced by other materials such as any suitable organic polymers or membranes that can be modified, immobilized, or self-assembled at the surface of electrodes **16** and doped with a ferrocene containing compound. Advantageously, the doped lipid membrane retains its biocompatible microenvironment in the presence of enzymes, thus the sensor is suitable for biochemical applications.

**[0053]** The coating may be permeable or impermeable, depending on the type of sensing mechanism to be employed. For example, a permeable coating may be used for amperometry-type sensors while an impermeable coating may be used for resistance or impedance-type sensors.

**[0054]** It is also possible to cover different electrodes **16** with different coatings, such as coatings comprising different materials or similar materials having different oxidization states.

**[0055]** In operation, electrochemical chip **10** is wired to external control and data taking instruments, through contact holes **24**. Reaction chamber **18** is filled with a liquid. The electrodes are biased in a typical manner for an electrochemical sensor or a sensor

array so that electrical signals can be detected to determine if, and/or how fast, a certain redox reaction occurs in the fluid. Thus, whether one or more particular target substances are present in the fluid, or the concentrations of the substances, can be determined. When required, one or both of an external counter electrode and an external reference electrode supported by cantilevers **20** may be used. Electrodes **16** may be all used as working electrodes. Or, one or more of electrodes **16** may be used as counter or reference electrodes.

**[0056]** As can be appreciated, electrochemical chip **10** is easy to fabricate. The two-plate structure makes it easy to form electrode array **16** since conducting layer **34** can be deposited onto a flat surface and then etched away to form individual electrodes. Similarly, other metal components such as bond pads and connection lines are easily formed. A large reaction chamber **18** is also possible, as it can be as deep as the full thickness of wafer **44**. Since two wafers are used, more electronic devices may be fit onto electrochemical chip **10** than onto a single wafer chip.

**[0057]** Conveniently, all electrodes, including working electrodes, counter electrodes, and reference electrodes, can be formed with the same material on the same conducting layer **34**. Optional counter electrodes and reference electrodes made of different materials may nonetheless be provided and supported by cantilevers **20**.

**[0058]** As all the electrodes **16** are located in one reaction chamber **18**, simultaneous sensing or testing is possible, which reduces sample usage and analyzing time. Multiple components of the same sample can be tested at the same time. Alternatively, data obtained from multiple electrodes can be combined to arrive at a more accurate or reliable results.

**[0059]** As can be appreciated, even without coating the electrodes with a coating doped with a ferrocene compound, forming an electrochemical chip as taught herein is still advantageous since the fabrication process is simple and inexpensive.

**[0060]** Electrochemical chip **10** may also be used in applications other than electrochemical sensing. For example, the electrodes can be used for AC or DC electrical measurements.

**[0061]** Other features, benefits and advantages of the present invention not expressly mentioned above can be understood from this description and the

accompanying drawings by those skilled in the art.

**[0062]** As can be understood by a person skilled in the art, many modifications to the exemplary embodiments of the invention described herein are possible. For example, conducting layer **34** (and hence electrodes **16**) may be made of any suitable metals or metal alloys such as Au, Pt, Ag, indium tin oxide (ITO), and the like, or conductive polymers. Insulating materials for layers **32**, **36** can be any suitable type of insulating polymers. Bonding material for layer **38** can be any suitable biocompatible and chemically resistive material.

**[0063]** Base plate **12** and cover plate **14** need not be chemically bonded. They can be simply stacked and mechanically held so that reaction chamber **18** is leak-proof, in which case bonding layer **38** may be omitted.

**[0064]** Further, cover plate **14** may have a window shape different from the one shown in **Figs. 2** and **4A-4D**. For instance, the window on the cover plate may have a shape other than a square when viewed from the front side. Further, the window on the cover plate need not be completely open. For example, **FIG. 5** illustrates another sensor array integrated electrochemical chip **10'**, wherein the opening in cover plate **14'** is etched only half-way through thus forming a depression. Cover plate **14** and base plate **12'** are bonded together to define a cavity, reaction chamber **18'**. Sample liquids can flow through channels **50** in cover plate **14'** into and out of reaction chamber **18'**. The entire backside of cover plate **14'** and the side walls of reaction chamber **18'** may be coated with a biocompatible material **38'** such as PDMS and the like.

**[0065]** Another alternative cover plate **14''**, which has no cantilever, is illustrated in **Fig. 6**. The silicon wafer of cover plate **14''** may be etched either from top or from bottom.

**[0066]** Electrodes **16** may also be formed on the side walls of reaction chamber **18**, if desired.

**[0067]** As illustrated in **Fig. 7**, electrodes **16'** may also be interconnected in parallel and grouped into two or more groups and each group of electrodes be connected to a common bond pad **52** through conducting lines **54**. As illustrated, each of the conducting lines extends from an electrode outwardly beyond a periphery of the electrode array.

**[0068]** If the base and cover plates are bonded with a bonding material that is not biocompatible, a layer of biocompatible, chemically inert material may be deposited on the side walls of the window of the cover plate before it is bonded to the base plate so as to provide biocompatible inner surfaces in the reaction chamber.

**[0069]** The invention, rather, is intended to encompass all such modification within its scope, as defined by the claims.